

# Molecular and cellular mechanisms of CLL: novel therapeutic approaches

Lisa Pleyer, Alexander Egle, Tanja Nicole Hartmann and Richard Greil

**Abstract** | The mainstay of therapy of chronic lymphocytic leukemia (CLL) is cytotoxic chemotherapy; however, CLL is still an incurable disease with resistance to therapy developing in the majority of patients. In recent years, our understanding of the biological basis of CLL pathogenesis has substantially improved and novel treatment strategies are emerging. Tailoring and individualizing therapy according to the molecular and cellular biology of the disease is on the horizon, and advances with targeted agents such as monoclonal antibodies combined with traditional chemotherapy have led to improved remission rates. The proposed key role of the B-cell receptor (BCR) in CLL pathogenesis has led to a number of possible opportunities for therapeutic exploitation. We are beginning to understand that the microenvironment is of utmost importance in CLL because certain T-cell subsets and stromal cells support the outgrowth and development of the malignant clone. Furthermore, an increase in our understanding of the deregulated cell-death machinery in CLL is a prerequisite to developing new targeted strategies that might be more effective in engaging with the cell-death machinery. This Review summarizes the progress made in understanding these features of CLL biology and describes novel treatment strategies that have also been exploited in current clinical trials.

Pleyer, L. *et al.* *Nat. Rev. Clin. Oncol.* 6, 405–418 (2009); published online 2 June 2009; doi:10.1038/nrclinonc.2009.72

## Introduction

The treatment of chronic lymphocytic leukemia (CLL) is in the process of substantially changing. While treatment approaches in the past were based on disease control that achieved a chronic indolent disease, treatment goals nowadays are aimed at achieving long-term remissions, at least in a less-indolent subset of patients. Such remissions are defined by negativity for minimal residual disease (MRD), as measured by highly sensitive methods such as multicolor flow cytometry. Achievement of an MRD-negative state is associated with superior long-term outcome. This has been shown for monotherapy with the monoclonal antibody alemtuzumab in pretreated patients with CLL (improved overall and treatment-free survival for MRD-negative patients);<sup>1</sup> the combination of fludarabine, cyclophosphamide and mitoxantrone as front-line treatment (longer response duration and overall survival for MRD-negative patients);<sup>2</sup> and first-line sequential<sup>3</sup> or combined therapy<sup>4</sup> with fludarabine, cyclophosphamide and rituximab (longer response duration for MRD-negative patients). Currently, cytotoxic drugs are still the mainstay of CLL therapy, with fludarabine being the backbone of many different treatment regimens. Although it is possible to achieve complete remission rates in the range of up to 80% using combinations of fludarabine with the anti-CD20 antibody rituximab in conjunction with cyclophosphamide and/or mitoxantrone,<sup>2,5</sup> approximately 20% of patients with CLL do not achieve complete disease control with

these conventional therapies. In this context it is important to emphasize that deactivation of the DNA damage pathway, most notably by loss of p53 function, leads to exquisite resistance to cytotoxic agents, including those mentioned above. Primary resistance to fludarabine, for instance, is almost uniformly observed in patients bearing p53-deletions<sup>6</sup> and has devastating consequences. Such patients survive for less than 1 year despite dose-intense salvage therapies. In addition, it is currently not possible to change the underlying biology of the disease by impressive reduction of cell numbers and/or lymph-node size alone, as risk factors such as immunoglobulin heavy-chain variable region gene (IgVH) mutational status are still able to predict progression-free survival, even in patients who achieve a complete remission.<sup>7</sup> In future it will be necessary to develop therapeutic strategies that circumvent cellular resistance mechanisms to cytotoxic agents, which are often caused by an inactive p53 pathway, in order to obtain sustained remission rates in patients with adverse prognostic factors. In an effort to address these medical needs, this Review focuses on the current knowledge of CLL disease biology, and how this increased understanding can be employed to develop therapeutic approaches that might directly modify central pathways of CLL maintenance and/or bypass cellular resistance mechanisms to cytotoxic drugs.

## Role of BCR-signaling pathways

Immunoglobulin diversity and, therefore, antigen receptor binding specificity of the B-cell receptor (BCR), results from the assembly of the variable (V), diversity

Laboratory for Immunological and Molecular Cancer Research and IIIrd Medical Department with Hematology, Medical Oncology, Hemostaseology, Rheumatology and Infectiology, Paracelsus Medical University Salzburg, Laboratory for Immunological and Molecular Cancer Research, Salzburg, Austria (L. Pleyer, A. Egle, T. N. Hartmann, R. Greil).

Correspondence: R. Greil, IIIrd Medical Department with Hematology, Medical Oncology, Hemostaseology, Rheumatology and Infectiology of the Paracelsus Medical University Salzburg, Laboratory for Immunological and Molecular Cancer Research, Muelner Hauptstrasse 4B, 5020 Salzburg, Austria [r.greil@salk.at](mailto:r.greil@salk.at)

## Competing interests

The authors declare no competing interests.

## Key points

- Antigenic input and B-cell receptor (BCR) signaling are important in the pathophysiology of chronic lymphocytic leukemia (CLL)
- A number of relevant signals downstream of the BCR signal, or that modify its signal, have been implicated in CLL and are currently exploited as targets for therapy
- The contribution of accessory immune cells and of stromal microenvironment to CLL pathophysiology is starting to be exploited in a therapeutic fashion
- CLL-specific proapoptotic and antiapoptotic influences contributed by microenvironmental and immunologic cues allow for a unique opportunity to target the core cell-death machinery in various combinations
- A large number of early clinical trials exploiting such rationales are currently under way and the results will need careful integration into future therapeutic concepts

(D) and joining (J) gene segments of the immunoglobulin (Ig) heavy chain (IgH) and the V and J segments of the Ig light chains. Further diversity arises from antigen triggered class switching recombination and the deletion or insertion of nucleotides, as well as the usage of different open reading frames. The third heavy chain complementary determining region (HCDR3) has the major role in determining antigen binding specificity,<sup>8</sup> and is created *de novo* by the VDJ recombination process.<sup>9</sup> Somatic hypermutation following mature B-cell stimulation with antigens may occur in a T-cell-dependent or T-cell-independent manner, mainly within germinal centers, but also outside classic germinal centers,<sup>10</sup> resulting in further antibody diversity.

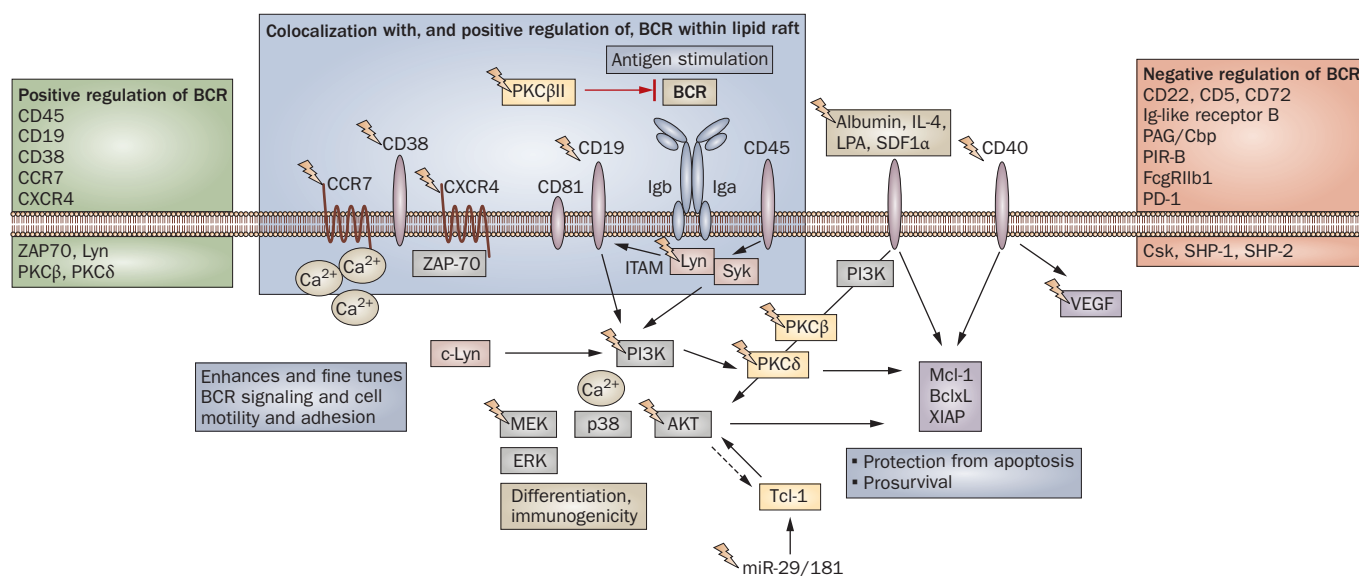
In CLL, the biased heavy chain Ig repertoire in conjunction with preferential usage of distinct light chain genes, as well as the use of similar amino acid motifs in the HCDR3, have led to the hypothesis of an antigen component being involved in the pathogenesis of the disease.<sup>11</sup> Clonal CLL selection by specific antigens is indicated by the high proportion of patients with CLL who express virtually identical Ig heavy and light chains, and the presence of precisely targeted somatic hypermutation patterns in the form of recurrent 'stereotyped' amino acid changes across the entire IgVH sequence.<sup>12,13</sup> Evidence for the important role of the BCR for CLL pathogenesis also stems from the fact that the mutational status observed in the BCR sequences is one of the strongest predictors of disease outcome.<sup>14,15</sup>

Initially it was assumed that CLL might comprise two different diseases: one with cells that express unmutated IgVH genes (U-CLL) derived from naive B cells, and another with mutated CLL cells (M-CLL) thought to be derived from antigen-experienced B cells. It is now known that all CLL cells bear the surface membrane phenotype of antigen experienced (CD27) and activated (CD23, CD25, CD69, CD71) B cells and show gene-expression profiles similar to memory B cells,<sup>16,17</sup> irrespective of IgVH mutational status. This finding is consistent with the notion that all cases of CLL have a common cellular origin and/or common mechanism of transformation<sup>16</sup> and a continued requirement for antigen after the transformation event in disease

perpetuation.<sup>18</sup> Comparative analysis of mutated and unmutated subgroups, however, suggests that B-CLL cells differing in IgVH phenotype may have different antigen encounter histories.<sup>18</sup> Specific stereotyped HCDR3s are preferentially associated with molecular, cytogenetic, phenotypic and clinical features, some of which portend a considerably more-aggressive disease with shorter time-to-initial treatment and worse overall survival times, and this might be regardless of IgVH mutation status.<sup>19,20</sup> This striking association between stereotyped BCRs and the phenotypic and clinical features for selected subsets of patients suggests that an antigen-driven process might be critical in determining clinical features and for modulating disease outcome, irrespective of mutation status in B-CLL.<sup>21</sup> In addition, several molecules that modify BCR signaling, such as ZAP-70 or CD38, display important prognostic power,<sup>22</sup> thus lending further support for the prominent role of BCR signaling in CLL pathophysiology, and identifying molecules involved in BCR signaling as important potential therapeutic targets.

Downstream signaling of the BCR in CLL is dominated by the kinases Lyn and Syk, which transduce survival and antiapoptotic signals after antigenic BCR triggering (Figure 1).<sup>23</sup> In CLL, antiapoptotic BCR signaling has been associated with prolonged activation of the MEK/ERK and PI3K/AKT pathways and with AKT-induced elevated expression of antiapoptotic Mcl-1, which leads to increased survival of malignant cells (Figure 1).<sup>24</sup> Signal transduction via Lyn is regulated and amplified via CD19 and these signals are responsible for the establishment of baseline signaling thresholds in B cells before antigen-receptor ligation, in addition to augmenting tonic signaling (that is, BCR signaling occurring independently of antigen-ligation) following BCR engagement.<sup>25</sup> Lyn was identified as a major contributor to antigen-independent BCR signaling as it is strongly overexpressed, constitutively active and aberrantly present in the cytosol.<sup>23</sup> Additionally, the recruitment and subsequent activation of Syk to immunoreceptor tyrosine-based activation motifs within the cytoplasmic tails of I $\alpha$  and I $\beta$  seems to be disturbed in CLL, as alternative transcripts of I $\beta$  have been described (Figure 1).<sup>26</sup> CLL clones with proliferative response to BCR ligation have considerably higher Syk levels than nonresponsive 'anergic' CLL cells,<sup>27</sup> and the tyrosine kinase ZAP-70 can partially restore BCR signaling when Syk is not expressed.<sup>28</sup>

CLL can be distinguished in two subsets depending on the incidence of somatic mutations in the IgV genes, with a poorer clinical prognosis for U-CLL compared with M-CLL.<sup>22</sup> ZAP-70, which is involved in T-cell-receptor signaling, is aberrantly expressed in some patients with CLL and shows partial, but not complete, overlap with overexpression of other risk factors such as the presence of CD38 or unmutated IgVH genes.<sup>29,30</sup> High ZAP-70 expression in B-CLL cells is associated with more-aggressive disease. While M-CLL cells are



**Figure 1** | The role of BCR signaling in the biology of CLL. The engagement of the BCR leads to complex formation of CD79alpha-beta heterodimers with several costimulatory molecules. This results in intracellular recruitment and activation of the protein tyrosine kinases Syk, ZAP-70 and Lyn at the immunoreceptor tyrosine-based activation motifs of the BCR and activation of several signal transducers and pathways such as p38, JNK, MEK/ERK and PI3K/PKC/AKT. Coligation of B cell negative regulatory molecules with the BCR results in phosphorylation of ITIMs (instead of ITAMs) and recruitment of SHP-1, which deactivates signal transduction molecules.<sup>140,141</sup> Positive regulators of the BCR are marked in green, while negative regulators are marked in red. The lightning bolt symbols indicate putative therapeutic targets. Abbreviations: BCR, B-cell receptor; Ig, immunoglobulin; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; LPA, lysophosphatidic acid; PD-1, programmed death 1; PIR-B, paired immunoglobulin-like receptor B; PKC, protein kinase C; SDF1α stromal-cell-derived factor 1α; SHP, Src homology 2 domain containing protein tyrosine phosphatase; Tcl-1, T-cell leukemia 1; XIAP, X-linked inhibitor of apoptosis protein.

considered energized, U-CLL cells seem to retain some capacity for competent BCR signaling, with an increased tendency to phosphorylate Syk and to recruit and phosphorylate ZAP-70.<sup>31</sup> The presence of ZAP-70 can enhance and prolong BCR signaling in CLL independent of its tyrosine kinase function, probably by acting as an adaptor protein.<sup>28</sup> The anergy of M-CLL cells is thought to be the result of chronic exposure to soluble (auto)antigens in the absence of co-stimulatory signals,<sup>32</sup> and of unresponsiveness to BCR signaling owing to receptor desensitization. In addition, BCR translocation to lipid rafts (that is, cholesterol-enriched microdomains in cell membranes) and associated signaling, differs in M-CLL compared with U-CLL. A constitutive exclusion of the BCR from lipid rafts was observed in M-CLL and might be responsible for impaired interactions between the BCR and the actin cytoskeleton.<sup>33</sup>

Another potential modifier of BCR signaling is CD38, which is a molecule that affects proliferation and longevity of the neoplastic clone.<sup>34</sup> CD38 ligation by monoclonal antibodies results in proliferation and blastic transformation of a subset of CLL cells, and engagement of CD38 as a receptor is a Lyn-dependent process.<sup>35</sup> Dynamic localization to lipid rafts (for example, mediated by CD31 crosstalk) and lateral association of CD38 with the BCR, CD19 and CD81 (Figure 1) was reported to be a prerequisite for CD38-mediated signaling, and

enhancement and fine-tuning of BCR signaling.<sup>36</sup> Importantly, ZAP-70 represents a limiting factor in the CD38 signaling pathway, probably serving as a cross-point where BCR signals are enhanced and where migratory signals from chemokine receptors intersect with growth signals mediated via CD38 (Figure 1).<sup>37</sup> This might be one reason why CD38<sup>+</sup>/ZAP-70<sup>+</sup> patients have a worse prognosis than CD38<sup>+</sup>/ZAP-70<sup>-</sup> patients.<sup>30</sup> However, functional linkage or a specific association with signaling capacity has not been proven so far, and elevated levels of CD38 and/or ZAP-70 may merely be markers of activation. In addition, the main determinant of BCR-mediated signaling may be the expression level of surface IgM, which is generally upregulated in U-CLL and downregulated to anergy in M-CLL.<sup>31</sup> This molecular signature of anergy is characterized by constitutive activation of MEK and ERK.<sup>38</sup> As CLL anergy primarily occurs in the mutated CLL subset, it is not surprising that ERK phosphorylation defines CLL cases with a favorable prognosis.<sup>38</sup>

### Targeting the BCR pathway

Pharmacological inhibition of BCR signaling could provide a dual therapeutic benefit, as disruption of both tonic and antigen-ligation-dependent BCR signaling is expected. In this respect, targets could be signaling components of the BCR itself or modifiers of these components. Agents that

**Table 1** | Agents targeting the BCR and downstream signaling pathways in CLL

Substance	Mechanism of action	Agent with additive or synergistic activity	Research status
Dasatinib	Lyn-inhibition, lower levels of Bcl-xL and Mcl-1, elevated p53	Fludarabine <sup>a</sup>	Cell lines and primary CLL cells <sup>39</sup>
Imatinib	Abl-inhibition	Chlorambucil <sup>b</sup>	<i>In vitro</i> <sup>132</sup>
NU7026	Kinase inhibitor	Chlorambucil <sup>b</sup>	<i>In vitro</i> <sup>43</sup>
Genistein	Kinase inhibitor, ZAP-70	Fludarabine	Primary CLL cells <sup>133</sup>
Dasatinib (and imatinib)	c-Abl-inhibition, prevent antiapoptotic CD40-program, restore drug sensitivity	Restores sensitivity to fludarabine, bortezomib, roscovitine, ABT737	Primary lymph node and peripheral blood CLL samples <sup>105</sup>
PP2 SU6656	Lyn inhibition	ND	<i>In vitro</i> , primary CLL cells <sup>23</sup>
U-0126 PD-98059	MEK1 inhibitor	Purine analogs 2-Cda <sup>a</sup>	EHEB CLL cell line, primary CLL cells <sup>54</sup>
LY294002	PI3K-inhibitor	ND	<i>In vitro</i> , primary CLL cells <sup>48</sup>
LY333531	PKC $\beta$ inhibitor	ND	<i>In vitro</i> , primary CLL cells <sup>48</sup>
Bis I	PKC inhibitor (classic and novel isoforms)	PI3K-inhibitor <sup>a</sup>	<i>In vitro</i> , primary CLL cells <sup>48</sup>
Staurosporine UCN-01	PKC inhibitor (nonselective)	ND	Phase I/II in CLL, <i>in vitro</i> , primary CLL cells <sup>45</sup>
PKC412	PKC inhibitor (classic isoforms)	ND	<i>In vitro</i> , primary CLL cells and cell lines <sup>134</sup>
Bryostatin-1	PKC modulator	Rituximab <sup>a</sup> , fludarabine <sup>a</sup>	Phase II in CLL <sup>45,51,135,136</sup>
Rottlerin	PKC $\delta$ and PKC $\theta$ inhibitor, reduction of Mcl-1 and XIAP	Vincristine <sup>b</sup>	Primary CLL cells <sup>49</sup>
AP0866	Inhibits biosynthesis of NAD <sup>+</sup> (involved in DNA-repair)	ND	Phase I/II in refractory CLL <sup>137</sup>
GSK690693	AKT	ND	Phase I in hematological malignancies <sup>138</sup>
Plerixafor (AMD3100), T140, TC14012, TN14003	Inhibitors of SDF-1/CXCR4 signaling, inhibits stromal mediated protection	Fludarabine <sup>a</sup>	Phase I/II in CLL Phase II in other NHL, <i>in vitro</i> , primary CLL samples <sup>86</sup>
TCP168, AMD3465	Inhibitors of SDF-1/CXCR4 signaling, inhibits stromal mediated protection	Fludarabine <sup>a</sup>	<i>In vitro</i> , primary CLL samples <sup>139</sup>
Anti-CCR7-Ab	Anti-CCR7-Ab potential blockage of CLL entry into secondary lymphoid tissue	ND	<i>In vitro</i> , primary CLL samples <sup>88</sup>

<sup>a</sup>Agents with additive activity. <sup>b</sup>Agents with synergistic activity. Abbreviations: Ab, antibody; BCR, B-cell receptor; CLL, chronic lymphocytic leukemia; ND, not determined; NHL, non-Hodgkin lymphoma; SDF-1, stromal-cell-derived factor 1; XIAP X-linked inhibitor of apoptosis protein.

target the BCR and current clinical trials are listed in Tables 1 and 2. Potential therapeutic targets in CLL are Lyn and Syk.<sup>32</sup> Specific inhibition of Lyn and Syk induced CLL-cell apoptosis *in vitro*, which indicates that these kinases primarily transmit prosurvival signals.<sup>32</sup> Inhibition of Lyn might partially 'mimic' an anergic state, as is present in M-CLL. The Abl and Src-kinase inhibitor dasatinib induces apoptosis in primary CLL cells *in vitro*, with a preference for the U-CLL and/or ZAP-70-bearing subgroups, at concentrations clinically achievable by oral administration.<sup>39</sup> Preliminary data of a currently ongoing phase II clinical trial of dasatinib in heavily pretreated patients with relapsed CLL was presented at the American Society of Hematology Annual Meeting and Exposition 2008. Data from 11 evaluable patients indicates that dasatinib monotherapy produced no complete or partial responses

according to National Cancer Institute Working Group (NCI-WG) criteria. However, several patients showed evidence of biologic activity of dasatinib, including partial responses in lymph nodes but worsening anemia, decline in stable disease and reductions of lymphocyte counts.<sup>40</sup> Although ZAP-70 and Syk are highly homologous structurally and functionally, direct inhibition of ZAP-70 by dasatinib is unlikely, as the binding affinity for Syk is tenfold higher than for most Src family kinases.<sup>39</sup> A specific targeting of ZAP-70 positive CLL, however, was suggested by inhibition of HSP-90 (heat shock protein 90).<sup>41</sup>

Decreased signaling via survival-signaling cascades involving AKT and ERK, reduced expression of anti-apoptotic Mcl-1 and Bcl-xL, and increased p53 levels are at the basis of the antiproliferative and proapoptotic capacity of dasatinib, probably as the result of inhibition

**Table 2** | Ongoing and currently recruiting clinical trials targeting BCR regulatory molecules

Substance	Drug target	Study phase	Disease entity	Clinical Trials identifier	Status
MDX-1342	Anti-CD19	I	CLL <sup>a</sup>	NCT00593944	Recruiting
Blinatumomab MT103	Anti-CD19/CD3	I II	Relapsed NHL ALL	NCT00274742 NCT00560794	Recruiting Recruiting
Y-90 BU12	Anti-CD19	I	CLL <sup>a</sup> , ALL <sup>a</sup>	NCT00643240	Recruiting
Combotox	Anti-CD19/CD22 immunotoxin	I	B-ALL	NCT00450944	Recruiting
Ofatumumab HuMax-CD20 <sup>TM</sup>	Anti-CD20 + CF ± chlormabucil	I/II III I II III	CLL <sup>a</sup> CLL <sup>b</sup> CLL <sup>a</sup> , FL Untreated CLL Untreated CLL	NCT00093314 NCT00349349 NCT00742144 NCT00410163 NCT00748189	Completed Recruiting Recruiting Ongoing Not yet open
Epratuzumab	Anti-CD20 and rituximab	III II II	Low grade NHL <sup>c</sup> Low grade NHL <sup>a</sup> Untreated FL	NCT00022685 NCT00044902 NCT00553501	Ongoing Completed Recruiting
90Y-hLL2	Anti-CD20 and autologous stem-cell transplant	I/II I/II	B-NHL <sup>a</sup> B-NHL <sup>a</sup>	NCT00421395 NCT00004107	Completed Ongoing
Veltuzumab hA20 IMMU-106	Anti-CD20	I/II I/II I/II	Untreated CLL NHL <sup>c</sup> NHL	NCT00546793 NCT00596804 NCT00285428	Recruiting Completed Completed
Inotuzumab Ozogamazin CMC-544	Anti-CD22 + rituximab <sup>d</sup> + rituximab vs R-CVP/R-FND	I/II I III	FL, DLBCL <sup>a</sup> B-NHL FL	NCT00299494 NCT00724971 NCT00562965	Recruiting Recruiting Recruiting
Galiximab IDEC-114	Anti-CD80 + rituximab	I/II I/II	FL <sup>a</sup> FL <sup>a</sup>	NCT00575068 NCT00048555	Completed Completed
Lumiliximab IDEC-152	Anti-CD23 + FCR + FCR	I I/II II/III	CLL <sup>a</sup> CLL <sup>a</sup> CLL <sup>a</sup>	NCT00046488 NCT00103558 NCT00391066	Completed Ongoing Recruiting
Apolizumab HU1D10	Anti HLA-DR + rituximab	II I II	B-NHL <sup>a</sup> LL, B-NHL CLL/SLL <sup>a</sup>	NCT00055783 NCT00029367 NCT00089154	Completed Completed Ongoing
Milatuzumab hLL1	Anti-CD74	I I/II	CLL, B-NHL <sup>e</sup> CLL, B-NHL <sup>e</sup>	NCT00504972 NCT00603668	Recruiting Recruiting
SGN-40	Anti-CD40	I/II	CLL <sup>a</sup>	NCT00283101	Completed
HCD122	Anti-CD40	I I/II	CLL <sup>a</sup> NHL, Hodgkin	NCT00108108 NCT00670592	Terminated Recruiting
HuMax-CD38	Anti-CD38	I/II	Myeloma	NCT00574288	Recruiting
Plerixafor	CXCR4 (SDF-1)	I/II	CLL	NCT00694590	Recruiting

<sup>a</sup>Advanced, relapsed or refractory disease. <sup>b</sup>Patients who did not respond to fludarabine and campath. <sup>c</sup>Patients who did not respond to rituximab. <sup>d</sup>Patients must have had at least 1 prior dose of rituximab and may not be refractory to rituximab. <sup>e</sup>Patients progressed after at least 1 prior treatment. Abbreviations: ALL, acute lymphoblastic leukemia; B-NHL, B-cell non-Hodgkin lymphoma; CF, endoxan and fludarabine; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FCR, fludarabine, cyclophosphamide and rituximab; FL, follicular lymphoma; NHL, non-Hodgkin lymphoma; R-CVP, rituximab, cyclophosphamide, vincristine and prednisone; R-FND, rituximab, fludarabine, novantrone and dexamethasone; SDF-1, stromal-cell-derived factor 1; SLL, small lymphocytic lymphoma.

of Lyn. An additive effect can be achieved by combination with fludarabine.<sup>39</sup> *Ex vivo*, CLL lymph-node samples display strong ERK activation together with high levels of Bcl-xL and Mcl-1, and this has been attributed to CD40-triggered events by interaction of CLL cells with the microenvironment.<sup>42</sup> Dasatinib and imatinib prevented antiapoptotic CD40 signaling and restored CLL drug sensitivity to fludarabine, bortezomib and roscovitine, indicating that chemoresistant niches, such as lymph nodes, may be sensitive to *c-Abl* inhibitors.<sup>42</sup> Importantly, these effects were also observed in the presence of p53 dysfunction. Thus, dasatinib can overcome chemoresistance, resulting in the re-establishment of sensitivity to p53-dependent (fludarabine) and p53-independent drugs (for

example, roscovitine, bortezomib, ABT-737).<sup>42</sup> Although limited effects of dasatinib as a single agent were observed in a phase II trial, a reduction of lymph-node size was observed in a major fraction of patients<sup>43</sup> and at least two further phase II clinical trials are currently ongoing (NCT00364286 and NCT00438854). Histone deacetylase inhibitors seem to be potential combination partners for dasatinib as they can directly repress the transcription of Lyn and other Src tyrosine kinases.<sup>44</sup>

Protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K)-mediated signaling pathways are centrally involved in controlling apoptosis and CLL-cell survival.<sup>45</sup> Approaches that target several isoforms of PKC are under clinical investigation. Several PKC isoforms are

constitutively active in CLL.<sup>46</sup> Although some PKC isoforms are key mediators of BCR signaling (for example, PKC $\beta$ II, which is overexpressed in CLL and inversely correlates with CLL-cell response to BCR engagement),<sup>47</sup> other isoforms induce AKT activation independently of BCR ligation and PI3K in CLL cells, but not in normal B cells, a fact that could be exploited in the future for producing a more targeted and selective therapy.<sup>48</sup> PKC $\delta$  is permanently activated and downstream of the constitutively activated PI3K in CLL. Specific blockade of PKC $\delta$  by rottlerin induces apoptosis and synergizes with vincristine in CLL cells but not in normal B lymphocytes.<sup>49</sup> It is possible that this synergistic effect of rottlerin is sufficient to disrupt the balance between antagonizing PKC $\alpha$  and PKC $\delta$  isoforms.<sup>49,50</sup> The PKC modulator, bryostatin 1, increases CD20 expression via MEK1/ERK signaling in a PKC-dependent manner in CLL B-cells, and leads to a twofold increase in apoptosis induction by rituximab.<sup>51</sup> Of interest, rituximab achieves inhibition of the Raf/MEK/ERK signaling pathway, resulting in Bcl-xL downregulation and chemosensitization.<sup>52</sup> Intriguingly, farnesyl transferase inhibitors, known to block Ras activity and ERK phosphorylation, have been shown to induce CLL cell apoptosis in primary CLL cells derived from patients with cladribine and fludarabine refractory disease.<sup>53</sup> Inhibition of MEK significantly enhances cytotoxicity of purine analogs in a CLL cell line.<sup>54</sup> However, although considerable, the toxicity of specific MEK inhibition alone is low. The sensitization of MEK to various cytotoxic agents is thought to result from a lower apoptotic threshold because of downregulation of downstream ERK targets such as Bcl-2, Bcl-xL and/or inhibitor of apoptosis proteins (IAPs).

Taken together, although defining the biological role of the BCR in CLL pathogenesis still remains a challenge, BCR-targeting strategies show promise as potential therapeutic approaches. The remarkable difference in clinical behavior between U-CLL and M-CLL is likely to involve BCR signal competence; thus, BCR-targeted treatment strategies will have to be carefully tailored and individualized to the molecular state of the patient.

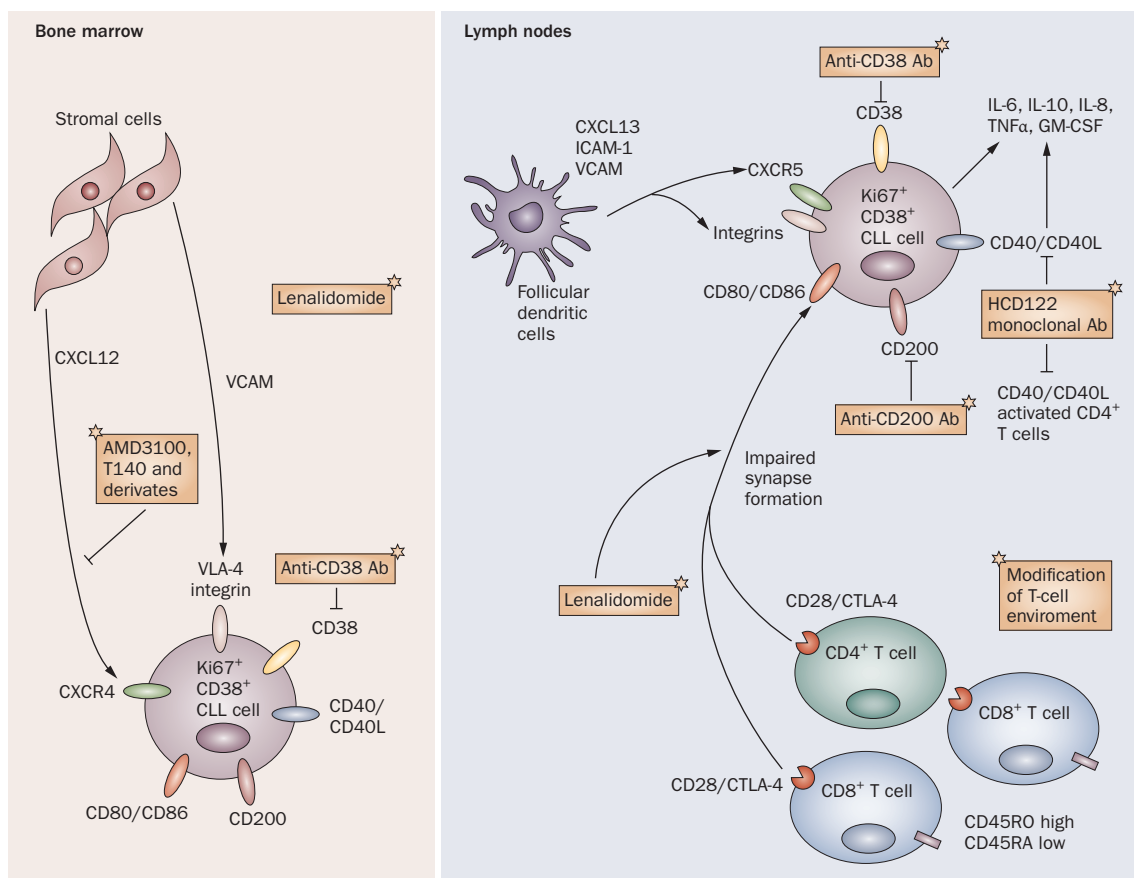
### Impact of the microenvironment

The major relevant events in CLL pathogenesis—proliferation, survival and hematogenous spread of the malignant cells—are dependent on specific combinations of cell types and soluble factors present in microenvironmental niches.<sup>55–58</sup> Imbalances in the composition of immune system components contribute to the creation of these niches. Several aberrant cytokine networks and cell–cell interactions have been identified (Figure 2)<sup>59,60</sup> that result in T-cell dysregulation, which is responsible for cell-death evasion and progressive accumulation of the malignant B cells. On the basis of a better understanding of underlying molecular mechanisms, targeting the microenvironment by either changing the cytokine networks or modulating immune effector cells, or both, is being considered as a novel therapeutic concept in CLL.

### Immune defects and the role of T cells

There is general agreement that patients with CLL are immunocompromised with defective T-cell, natural-killer-cell and dendritic-cell function, in addition to the obvious B-cell defects.<sup>55,61</sup> Patients with CLL typically develop hypogammaglobulinemia and progressive immune deficiency, which impairs their immune response to vaccines.<sup>62</sup> Rather surprisingly, absolute T-cell numbers are increased and the peripheral blood CD4<sup>+</sup> and CD8<sup>+</sup> T-cell repertoire is markedly oligoclonal.<sup>63</sup> The increase in T-cell numbers is mainly because of an increased CD8<sup>+</sup> subpopulation, which results in a lower CD4<sup>+</sup>:CD8<sup>+</sup> ratio, especially in patients with progressive disease.<sup>64,65</sup> Remarkably, Mackus and colleagues reported that only cytomegalovirus-positive patients with CLL displayed increased numbers of CD8<sup>+</sup> cells with a CD45RA<sup>+</sup>CD27<sup>-</sup> cytotoxic phenotype directed at cytomegalovirus, suggesting that repeated antigenic stimulation *in vivo* by viral infections can contribute to the disturbed immune balance in CLL.<sup>57</sup> Nevertheless, adoptive immunotherapy experiments with cytotoxic T-cell lymphocytes (CTLs) turned out to be disappointing, as they resulted in rather weak antitumor responses despite successful CTL production.<sup>66</sup> This gave rise to the search of additional mechanisms of how CLL cells are capable of escaping immune surveillance. Implicated in the abnormal immune function are immunosuppressive factors and an acquired functional deficiency of the CD40 ligand (CD40L).<sup>66</sup> CLL cells are particularly inept at antigen presentation, probably because of the low expression of immune co-stimulatory and adhesion molecules.<sup>67</sup> The capability of immune synapse formation between CLL and T cells is highly impaired, and CLL T cells display reduced synapse-related signaling and proliferative capacity.<sup>68</sup> Most interestingly, direct-contact coculture of CLL B cells with heterologous healthy T cells induced these defects in the healthy T cells as well.<sup>68</sup>

In CLL not only are the CD4:CD8 ratios imbalanced but there is an additional shift towards activated effector/memory T cells in both CD4<sup>+</sup> and CD8<sup>+</sup> subpopulations.<sup>69</sup> Furthermore, expression of CTLA-4, which has the opposite effect to the co-stimulatory molecule CD28, is reduced in CLL T cells,<sup>70</sup> and CTLA-4 knockout mice develop a clinical and pathological syndrome similar to human CLL.<sup>71</sup> One means by which immunogenicity could be increased in CLL is by manipulation of the CD40/CD40L pathway. Activation of CD40 by CD40L induces proliferation and rescues the cells from spontaneous and chemotherapy-induced apoptosis. CD40 activation also induces secretion of cytokines such as interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-alpha, IL-8, and granulocyte-macrophage colony-stimulating factor. HCD122, a novel anti-CD40 monoclonal antibody, was shown to inhibit CD40L-induced activation of signaling pathways, proliferation and survival, and secretion of cytokines, and turned out to be a potent mediator of antibody-dependent cellular cytotoxicity, lysing CLL cells more efficiently than rituximab



**Figure 2** | Cellular interactions as potential therapeutic targets in the CLL microenvironment. The diagram shows the crosstalk of CLL cells with accessory cells in the bone marrow and lymph-node microenvironment, and potential therapeutic targets. Agents targeting specific crosspoints of the interactions are marked with stars. In the proliferation centers the growth of leukemic cells is favored by an advantageous T-cell help. Selective manipulation of T-cell subsets or cytokine networks involved in this interaction can be therapeutically exploited, potential agents are anti-CD200 or HCD122 antibody or lenalidomide. CLL cell interaction with stromal cells and follicular dendritic cells via chemokine receptors and integrins leads to extended survival. Chemokine receptor antagonists such as AMD3100 (targeting the CXCR4–CXCL12 interaction) and anti-CD38 antibodies possibly interrupt this survival support. Note that interactions and possible therapeutic agents displayed in a certain organ are dominantly present in this organ, but not necessarily restricted to it. Abbreviations: Ab, antibody; CLL, chronic lymphocytic leukemia; GM-CSF, granulocyte macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; IL, interleukin; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; VCAM, vascular cell adhesion molecule.

*in vitro*.<sup>72</sup> Conversely, CD40L expression via gene therapeutic approaches has been proposed as a therapeutic concept.<sup>73</sup> One important downstream molecule to augment the proliferation and survival of memory CD4<sup>+</sup> T cells is OX40L. Transfer of a combination of CD40L and OX40L molecules in a fibroblast transference technique induced a cytotoxic T-cell immune response against autologous tumor cells, which could potentially be used to generate immunogenic tumor cells for active or adoptive immunotherapy of CLL.<sup>74</sup>

A striking presence of CD4<sup>+</sup>CD40L<sup>+</sup> T cells is observed in pseudofollicles, the hypothesized proliferative compartment and histopathologic hallmark of CLL present in lymph nodes and to a lesser extent in the bone marrow. Pseudofollicles are not only a collection of proliferating monoclonal CD5<sup>+</sup> B lymphocytes but are also formed by a number of bystander nontumor cells. In pseudofollicles,

proliferative prolymphocytes and paraimmunoblasts are found to be interspersed with T lymphocytes, which are supposed to provide short-term proliferative support to the malignant cells. Most T lymphocytes found in these centers belong to the CD4<sup>+</sup> subset and are in close contact with proliferating Ki67<sup>+</sup> CLL cells.<sup>75</sup> Several CD4<sup>+</sup> T cells within pseudofollicles express CD40L, implying that they are in an activated state. Furthermore, follicular dendritic cells (FDCs) may be detected within pseudofollicles,<sup>76</sup> which is reminiscent of the classical copresence of FDCs and Ag-specific CD4<sup>+</sup> cells in the germinal centers of secondary lymphoid follicles. It is unclear how CD4<sup>+</sup> T cells in CLL pseudofollicles acquire the expression of CD40L. In individual CD38 bimodal patients (that is, patients with two distinct CLL cell populations, one CD38<sup>+</sup> and one CD38<sup>-</sup>), the CD38<sup>+</sup> subpopulation in bone marrow was higher than in the peripheral blood, which suggests

that interaction with the bone marrow environment might influence the expression of CD38.<sup>77,78</sup> CD38 expression is generally higher in areas of pseudofollicles, and proliferative markers such as Ki-67 might change upon contact with activated CD4<sup>+</sup> T cells. In addition, an important role for CD38 in CD8<sup>+</sup> cells can be inferred from the prognostic potential CD38 expression displayed in male patients with CLL.<sup>79</sup> Currently, a phase I/II clinical trial is underway testing an anti-CD38 antibody (HuMax-CD38™, Genmab, Copenhagen, Denmark) in patients with multiple myeloma (ClinicalTrials identifier NCT00574288). In CLL lymph nodes, Ki-67<sup>+</sup> CLL cells were more likely to be in direct contact with CD4<sup>+</sup> activated T-helper lymphocytes than Ki-67<sup>-</sup> cells.<sup>78</sup> The strong implication of T-cell imbalances in CLL pathogenesis gives rise to a number of novel therapeutic targets. One potential target is CD200, which is an immunomodulatory molecule that is overexpressed in CLL and has been implicated in the induction of regulatory T cells and downregulation of Th1 immune responses.<sup>81</sup> The combination of an immune induction paralleled by a disruption of immunosuppressive factors makes CD200 blockage, (for example, with an inhibiting antibody) a potential powerful tool for future treatment of CLL.<sup>81</sup>

#### Other supportive cellular networks

Apart from the activated T lymphocytes that are supposed to provide short-term proliferative support, a number of other accessory cell types can be found in the CLL microenvironment. These accessory cells are in close contact with the accumulating leukemic pool and provide long-term support and survival benefits. *In vitro*, CLL cells derive survival benefits from interactions with accessory cells, such as marrow stromal cells.<sup>82</sup> Stromal cells support the survival of CLL cells not only by direct integrin-mediated adhesion to the stroma but also by the secretion of soluble factors such as chemokines. Expression of the major lymphocyte integrin ligands VCAM-1 (vascular cell adhesion molecule 1) and ICAM-1 (intercellular adhesion molecule 1) that bind the integrins VLA-4 (also known as integrin alpha-4) and LFA-1 (also known as integrin alpha-L), respectively, was also reported on FDCs (Figure 2).<sup>83</sup> Furthermore, FDCs produce the B-cell chemokine CXCL13, which is responsible for the localization of B lymphocytes within B-cell follicles of secondary lymphoid tissues.<sup>84</sup> CXCR5, the chemokine receptor for CXCL13, is highly expressed on CLL cells.<sup>85</sup> Other homeostatic chemokine receptors such as CCR7 and CXCR4 are highly expressed in CLL. In addition, marrow stromal cells and extramedullary stromal cells of mesenchymal origin secrete high amounts of stromal-cell-derived factor 1 (SDF-1, CXCL12), the ligand of CXCR4. VCAM and CXCL12, secreted by marrow stromal cells, have vital roles during early, normal B-cell development and CLL cells are rescued from apoptosis by cell–cell contact with these cells.<sup>59,86</sup> In addition, VEGF, which is important for microvessel formation in the CLL microenvironment, has a growth

promoting and survival effect on CLL cells, and the motility of CLL cells within certain niches seems to be dependent on VEGF interaction with integrin signaling.<sup>87</sup> Targeting CXCR4, CCR7 and/or their interaction with ligands has been used as a novel tool for treatment, and is currently being explored extensively *in vitro* and in clinical trials (Tables 1 and 2).<sup>86,88</sup>

#### Targeting the CLL microenvironment

Rapidly emerging knowledge on microenvironmental influences provides a rationale for therapeutical use of immunomodulating agents in CLL treatment. Lenalidomide, which is already approved for use for other tumors, and actimid (pomalidomid), which is not approved yet, are closely related to thalidomide, but less toxic. Lenalidomide and actimid have shown promising antineoplastic activity in various tumor types, including multiple myeloma, myelodysplastic syndrome, renal-cell carcinoma, and prostate cancer.<sup>89,90</sup> Our understanding of the mechanisms of action of these drugs is still in its infancy but they clearly change the angiogenesis and the cytokine micromilieu, and modulate the immune system by enhancing cytotoxic T-cell and natural-killer-cell activity. As lenalidomide does not show an antiproliferative effect *in vitro* but a clear antitumor effect *in vivo*, its efficacy seems to be directly dependent on microenvironmental factors. Interestingly, lenalidomide was recently shown to improve the above discussed impaired synapse formation between CLL and T cells.<sup>68</sup> Furthermore, both thalidomide and lenalidomide downregulate VEGF, IL-6, and TNF-1.<sup>91</sup> Lenalidomide is clinically active as a single agent in patients with relapsed or refractory CLL.<sup>92,93</sup> Although the exact lenalidomide dose is still under evaluation owing to tumor-flare reactions seen with very high doses,<sup>94</sup> the findings nevertheless set the stage for future clinical studies of lenalidomide and related immunomodulatory drugs, not only as single agents, but also as modulators of established chemotherapy regimens or maintenance treatments.

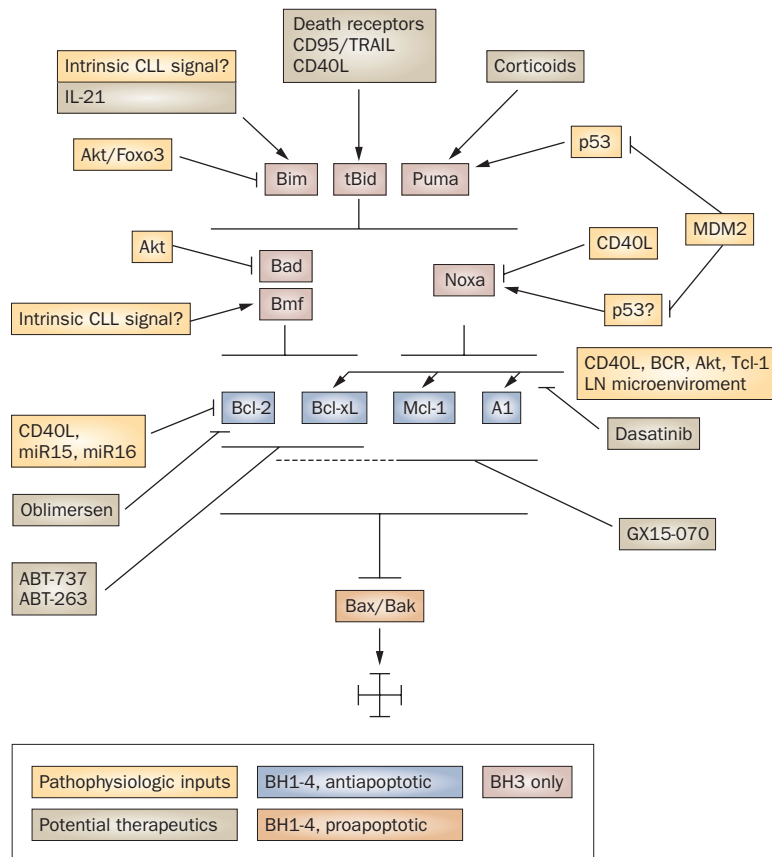
#### Aberrant apoptotic signaling pathways

Multiple signals converge in CLL cells to influence cellular fate. Cell death is regulated by a network of cellular signaling cascades that are also influenced by intracellular sensor modules.<sup>59</sup> A shortcut to cell death is present in the form of death receptors; in CLL they are CD95/Fas and TRAIL (tumor-necrosis factor-related apoptosis-inducing ligand). Upon ligation, these receptors create a platform for activation of initiator caspases that directly feed into a caspase cascade leading to cell death.<sup>95</sup> Such 'shortcut' killing potential is tightly regulated. The intrinsic cell death pathway, or mitochondrial pathway, is regulated by the balance between antiapoptotic and proapoptotic members of the Bcl-2 family. Antiapoptotic proteins (Bcl-2, Bcl-xL, Bcl-w, A1, Mcl-1) possess the Bcl-2 homology (BH) domains BH1–4, whereas proapoptotic proteins (Bax, Bak) contain domains BH1–3, and the so-called 'BH-3 only' proteins contain exclusively the BH3 domain.

Non-death-receptor transmitted death signals derive from developmental cues (for example, Bim-dependent B-cell killing upon BCR crosslinking) or from a lack of survival signals.<sup>96</sup> Such death signals also originate in sensor platforms, such as the DNA damage sensor network involving the ATM (ataxia telangiectasia mutated) and p53 tumor suppressors, which prominently determine survival and treatment outcomes in CLL. All these pathways modify the action of death sensory molecules, the most important of which are called “BH3-only” proteins (for example, Bim, Bid, Bmf, Puma, Bad and Noxa)—a name derived from their short homology with members of the classical Bcl-2 family of cell-death regulators. The BH3-only proteins are regulated by incoming cell death and survival signals and transmit these signals to the core machinery made up of proapoptotic effector molecules Bax and Bak as well as of antiapoptotic Bcl-2 family members (for example, Bcl-2 itself, Bcl-xL, Mcl-1 and A1). In one model, BH3-only proteins counteract the present prosurvival Bcl-2-type proteins in order to overcome this cell-death inhibition and initiate the Bax/Bak signal to initiate a caspase cascade.<sup>96</sup> Although a few BH3-only proteins (for example, Bim) can counteract all known antiapoptotic Bcl-2 family members, others, such as Bmf or Noxa, can only counteract some of the opponents within the family (Figure 3). Thus, a complex network is formed by which the cell establishes a net signal that will determine its fate.

A number of death signaling components have been characterized in CLL. CLL cells express CD95 but are largely resistant to CD95 crosslinking.<sup>97</sup> Overexpression of the CD95 regulator TOSO in CLL might contribute to CD95 resistance.<sup>98</sup> Although CD40 signaling upregulated CD95 in CLL, cells remained resistant to CD95 triggering.<sup>99</sup> This effect, however, was transient in a subset of patients where CD95 sensitivity was attained after 72 h.<sup>99</sup> IL-15 treatment was also able to sensitize CD40-stimulated CLL cells to CD95 crosslinking.<sup>100</sup> In addition, CD40L-stimulated CLL cells were rendered CD95-sensitive by XIAP (X-linked inhibitor of apoptosis protein) inhibition using non-SMAC (second mitochondria-derived activator of caspases) mimetic, synthetic compounds.<sup>101</sup> Also, CD40-activated CLL cells were effectively killed by genetically engineered effector cells expressing both the CD95 ligand (CD178) and TRAIL.<sup>102</sup> With regard to TRAIL sensitivity, the *ex vivo* phenotype of CLL is again one of resistance.<sup>103</sup> However, two publications have shown that histone deacetylase inhibition is able to sensitize CLL to the effects of TRAIL and that the TRAIL receptor DR4, but not DR5, was involved in cell killing.<sup>103,104</sup>

As mentioned above, CD40 has a prominent role in CLL pathogenesis. In terms of cell-death regulation, CD40 signaling has been suggested to trigger a major reprogramming along the Bcl-2 governed pathway to cell death.<sup>105–107</sup> In parallel to a pattern observed in CLL from lymph-node tissue, CD40L triggered upregulation of Mcl-1 and A1, as well as Bcl-xL antiapoptotic proteins.<sup>106</sup> In one report this triggered upregulation also



**Figure 3** | Therapeutic roads into the Bcl-2 death-signaling machinery. The core of the figure represents the interactions between BH3-only proteins with respect to their specific inhibition of antiapoptotic multidomain Bcl-2 family members. This inhibition then leads to an activation of the cell-death executioner machinery. The outer circle of the figure represents signals that influence cell-death machinery—either as intrinsic signals or as therapeutic approaches. Abbreviations: BCR, B-cell receptor; BH1–4, Bcl-2 homology domain 1–4; BH3, Bcl-2 homology domain 3; CD40L, CD40 ligand; CLL, chronic lymphocytic leukemia; IL, interleukin; LN, lymph node; Tcl-1, T-cell leukemia-1; TRAIL, tumor-necrosis-factor apoptosis-inducing ligand.

led to downregulation of the BH3-only protein Noxa, while the relatively highly expressed Bim and Bmf remained (almost) unchanged;<sup>106</sup> however, in another report Bim was reduced.<sup>105</sup> In CLL, Noxa did not seem to be exclusively induced by p53, but regulated by extracellular signaling.<sup>108,109</sup> Among the other BH3-only proteins, Puma has been shown to be regulated in response to p53 stimuli,<sup>108</sup> and Bim and Bmf seem to be constitutively expressed.<sup>110</sup> Other upstream signals involved in regulation of Bcl-2 family member proteins in CLL include extracellular cues (such as BCR or cytokine signaling), microRNA input or conventional therapeutic intervention via cytotoxic drugs.

BCR signaling has been reported to also be able to regulate Mcl-1.<sup>24</sup> This was suggested to be mediated via Akt signaling. Akt has also been shown to target BH3-only proteins Bad and Bim for deactivation by phosphorylation.<sup>111,112</sup> Interestingly the Akt signal seems to be positively modified by the overexpression of Tcl-1, which is

**Table 3** | Ongoing and currently recruiting clinical trials targeting cell-death regulatory molecules

Substance	Study phase	Drug mechanism	Disease entity	Clinical Trials identifier	Status
GX15-070	I/II	BH3 mimetic	Untreated CLL	NCT00600964	Completed
GX15-070	I/II	BH3 mimetic	Pretreated CLL	NCT00438178	Completed
GX15-070, fludarabine and rituximab	I	BH3 mimetic	Pretreated CLL	NCT00612612	Recruiting
ABT-263	I/II	BH3 mimetic	Pretreated CLL	NCT00481091	Recruiting
ABT-263	I PK	BH3 mimetic	Pretreated CLL and others	NCT00743028	Recruiting
AT-101	II	BH3 mimetic	Pretreated CLL	NCT00286780	Completed
AT-101	II	BH3 mimetic	Pretreated CLL and others	NCT00275431	Completed
Oblimersen, fludarabine and rituximab	I/II	Bcl-2 antisense	Pretreated CLL	NCT00078234	Active, but not recruiting
Oblimersen	I/II	Bcl-2 antisense	Pretreated CLL	NCT00021749	Completed
Oblimersen, fludarabine and cyclophosphamide	I/II	Bcl-2 antisense	Pretreated CLL	NCT00024440	Active, but not recruiting
SPC2996	I/II	Bcl-2 antisense	Pretreated CLL	NCT00285103	Active, but not recruiting
AEG35156 <sup>a</sup>	I/II	XIAP inhibitor	Pretreated CLL	NCT00768339	Recruiting

<sup>a</sup>Drug target is inhibitor of apoptosis protein. Abbreviations: BH3, Bcl-2 homology domain 3; CLL, chronic lymphocytic leukemia; PK, pharmacokinetic; XIAP, X-linked inhibitor of apoptosis protein

found in a relatively large proportion of patients with CLL and has been proposed to be the consequence of a dysregulation of the microRNAs, miR-29 and miR-181.<sup>111,113</sup> In addition, miR-15 and miR-16 were proposed to be the basis of constitutive Bcl-2 overexpression in CLL.<sup>111,114,115</sup> Another reported signal that modifies cell-death machinery is IL-21, which mediates apoptosis through upregulation of Bim.<sup>116</sup> Increasing our understanding of the deregulated cell-death machinery in CLL is a prerequisite to developing new targeting strategies that might engage the cell-death machinery in a more-effective way.

### Targeting apoptotic pathways

Currently, the mainstay of therapeutic strategies that kill CLL cells is still cytotoxic chemotherapy with alkylating agents or purine analogs. The major contribution to cell death in this respect stems from the DNA damage response via p53 that leads to a dominant cell-death signal via Puma.<sup>108,117</sup> A major problem encountered with these strategies is that a number of patients with CLL harbor defects in the DNA damage machinery that leads to deactivation of the pathway. Apart from the prognostically highly significant del17p<sup>6</sup> (a surrogate marker for p53 deletion), p53 mutation,<sup>118</sup> ATM deletion or mutation<sup>119</sup> and changes in the MDM2 p53 signal modifier<sup>120</sup> have been described to influence prognosis and potentially therapy sensitivity by modulating the DNA damage response. The challenge thus seems to be to bypass such resistance and produce p53-independent cell death. In CLL without deficient DNA damage response, specific microenvironmental cues may protect a small population effectively from current death-inducing treatment strategies and thus harbor the seed of therapeutic failure.

In this respect, classic chemotherapy may still be part of a winning concept, if combined with modulation of the relevant cell-death pathways.

Regarding the targeting of death-receptor pathways, the exploitation of CD95 signaling seems to be restricted, as systemic CD95 triggering leads to fulminant liver toxicity.<sup>121</sup> The role of TRAIL receptor targeting is currently under development. While recombinant TRAIL is developed, a strategy of triggering TRAIL receptors via antibody-strategies is also pursued. However, no trials in CLL have been reported to [clinicaltrials.gov](http://clinicaltrials.gov). Efforts to combine TRAIL receptor agonists with histone deacetylase inhibitors will be important.<sup>103,104</sup>

Modification of the cell-death machinery along the CD40 signaling cascade seems promising. While CD154 (CD40L) has previously been used to generate relevant immune responses,<sup>73</sup> its application was able to induce the p53-related transcription factor p73, leading to a sensitization of p53-deficient CLL cells to conventional therapeutics such as fludarabine.<sup>122</sup> This result suggests that the CD40 signal may also have a positive effect on conventional therapy. On the other hand, inhibition of Abl and Src-like kinases by dasatinib was able to revert the complete antiapoptotic program induced by CD40 stimulation and may contribute to overcoming chemoresistance in microenvironmental niches.<sup>105</sup>

Finally, a number of approaches have been taken to directly modulate the core components of the Bcl-2 cell-death machinery. The Bcl-2 antisense molecule oblimersen is the most advanced agent in clinical testing. Encouraging phase I/II and phase III data in CLL were reported,<sup>123,124</sup> with longer response duration after fludarabine and cyclophosphamide plus oblimersen than

fludarabine and cyclophosphamide alone. Oblimersen has been filed for FDA approval and is the first agent to change the cell-death outcome in CLL by targeting the cell-death machinery. However, given the important roles of other pro-survival Bcl-2 family members such as Mcl-1 and A1 for the pathogenesis of CLL (discussed earlier), targeting of Bcl-2 alone might be less efficient than other targeting strategies in development. New classes of substances in development are the “BH3-mimetics” and “pan-Bcl-2 family antagonists”. These molecules mimic the BH3 domain of BH3-only death-inducing proteins and are thought to liberate BH3-only proteins from the inhibition by antiapoptotic Bcl-2 proteins, thus making them effective killers. A relevant therapeutic window seems to be achieved by the tumor cells themselves. Owing to oncogenic stress signals and failed checkpoint controls, tumor cells display a balance between proapoptotic and antiapoptotic signals that is much closer to the cell-death threshold than is the case in normal tissues, which makes tumors more susceptible to the effects of antiapoptotic protein signals.<sup>125</sup>

ABT-737<sup>126</sup> (plus orally available derivative ABT-263) and GX15-070 (Obatocox)<sup>127</sup> were described to kill target cells using a BH3-only protein displacement strategy. While ABT-737 shows “Bmf-like” specificity (that is, it targets Bcl-2 but not Mcl-1-like proteins), data presented for GX15-070 suggest a “Noxa-like” behavior (that is, it efficiently targets Mcl-1 but not Bcl-2).<sup>128</sup> Consequently, a combination of both substances was more effective than the single substance.<sup>128</sup> Both substances are in clinical trials in CLL, with a phase I trial in extensively pretreated disease reported for Obatocox<sup>129</sup> and a phase I/II study of ABT-263 published in abstract form.<sup>130</sup> Both substances showed single agent efficacy in extensively pretreated collectives. At least one further substance with Bcl-2 binding activity, AT-101, a gossypol derivative, is in development in CLL. Preclinical data suggest that AT-101 may help to overcome stroma-mediated drug resistance;<sup>131</sup> phase II data were reported in abstract form.<sup>41</sup> Trials underway are listed in Table 3.

Given the expression profile of Bcl-2 proteins in CLL cells, a number of strategies seem promising. For example, one might combine substances that selectively downregulate antiapoptotic Bcl-2, such as ABT-263, with substances that preferentially target antiapoptotic Mcl-1, such as GX15-070, to achieve additive or synergistic

effects. Other strategies could be aimed at upstream signals, such as targeting CD40 using CD40 antibodies or dasatinib. GX15-070 could be used in combination with oblimersen to cover a wider range of Bcl-2 family proteins. On a more conventional note, BH3-only mimetic treatment might help counteract microenvironmentally determined chemotherapy resistance in combination with cytotoxic CLL treatment.

## Conclusions

Currently, the major problem in the treatment of CLL is that, despite promising response rates in recent years, patients with high-risk disease still seem to relapse with mostly unchanged kinetics. During these relapses the disease is often refractory to conventional chemotherapeutics. Targeting the immune and micro-environmental interactions in CLL might provide the means to relieve protection of the malignant cells derived from those interactions, and may create strong synergies with conventional therapeutics, allowing control of CLL even in the ‘hideaway’ places where residual disease survives conventional therapeutics. In addition, chronic modulation of pathophysiologically relevant signals may modify the rate of disease progression. Such strategies may then help to maintain an MRD-negative state in CLL after intensive chemoimmunotherapy, a state that has been associated with superior long-term survival in patients with CLL. Finally, a better understanding of the ways in which CLL cells escape cell-death cues from extracellular signals as well as from cytotoxic stress will facilitate the intelligent design of strategies employing the growing number of modulators available in this field.

## Review criteria

The information for this Review was compiled by searching the PubMed database for articles published before 10 November 2008, including electronic publications available ahead of print. In addition, selected topics were identified by searching the abstract databases of the American Society for Hematology and the ASCO websites. Search terms included “chronic lymphocytic leukaemia” “B cell receptor” “T cell,” “integrins” “stroma” “adhesion” “cell death” and “Bcl-2”. Full articles were obtained and references were checked for additional material and primary references when appropriate. Listings of ongoing clinical trials for CLL were retrieved from the [www.clinicaltrials.gov](http://www.clinicaltrials.gov) website.

- Moreton, P. *et al.* Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. *J. Clin. Oncol.* **23**, 2971–2979 (2005).
- Bosch, F. *et al.* Fludarabine, cyclophosphamide, and mitoxantrone as initial therapy of chronic lymphocytic leukemia: high response rate and disease eradication. *Clin. Cancer Res.* **14**, 155–161 (2008).
- Lamanna, N. *et al.* Sequential therapy with fludarabine, high-dose cyclophosphamide, and rituximab in previously untreated patients with chronic lymphocytic leukemia produces high-quality responses: molecular remissions predict for durable complete responses. *J. Clin. Oncol.* **27**, 491–497 (2009).
- Boettcher, S. *et al.* Quantitative MRD assessments predict progression free survival in CLL patients treated with fludarabine and cyclophosphamide with or without rituximab—a prospective analysis in 471 patients from the randomized GCLLSG CLL8 trial [abstract]. *Blood* **112**, a326 (2008).
- Keating, M. J. *et al.* Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J. Clin. Oncol.* **23**, 4079–4088 (2005).
- Dohner, H. *et al.* P53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. *Blood* **85**, 1580–1589 (1995).
- Byrd, J. C. *et al.* Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: justification for risk-adapted therapy. *J. Clin. Oncol.* **24**, 437–443 (2006).
- Xu, J. L. & Davis, M. M. Diversity in the CDR3 region of V(H) is sufficient for most antibody specificities. *Immunity* **13**, 37–45 (2000).

9. Maizels, N. Immunoglobulin gene diversification. *Annu. Rev. Genet.* **39**, 23–46 (2005).
10. William, J. *et al.* Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science* **297**, 2066–2070 (2002).
11. Chiorazzi, N. & Ferrarini, M. B cell chronic lymphocytic leukemia: lessons learned from studies of the B cell antigen receptor. *Annu. Rev. Immunol.* **21**, 841–894 (2003).
12. Messmer, B. T. *et al.* Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. *J. Exp. Med.* **200**, 519–525 (2004).
13. Murray, F. *et al.* Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. *Blood* **111**, 1524–1533 (2008).
14. Damle, R. N. *et al.* Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood* **94**, 1840–1847 (1999).
15. Hamblin, T. J., Davis, Z., Gardiner, A., Oscier, D. G. & Stevenson, F. K. Unmutated Ig V-H genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* **94**, 1848–1854 (1999).
16. Rosenwald, A. *et al.* Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J. Exp. Med.* **194**, 1639–1647 (2001).
17. Klein, U. *et al.* Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J. Exp. Med.* **194**, 1625–1638 (2001).
18. Damle, R. N. *et al.* B-cell chronic lymphocytic leukemia cells express a surface membrane phenotype of activated, antigen-experienced B lymphocytes. *Blood* **99**, 4087–4093 (2002).
19. Stamatopoulos, K. *et al.* Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. *Blood* **109**, 259–270 (2007).
20. Athanasiadou, A. *et al.* Recurrent cytogenetic findings in subsets of patients with chronic lymphocytic leukemia expressing IgG-switched stereotyped immunoglobulins. *Haematologica* **93**, 473–474 (2008).
21. Maurer, K. *et al.* Immunoglobulin gene segment usage, location and immunogenicity in mutated and unmutated chronic lymphocytic leukaemia. *Br. J. Haematol.* **129**, 499–510 (2005).
22. Hamblin, T. J. Prognostic markers in chronic lymphocytic leukaemia. *Best Pract. Res. Clin. Haematol.* **20**, 455–468 (2007).
23. Contri, A. *et al.* Chronic lymphocytic leukemia B cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. *J. Clin. Invest.* **115**, 369–378 (2005).
24. Longo, P. G. *et al.* The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells. *Blood* **111**, 846–855 (2008).
25. Fujimoto, M., Poe, J. C., Jansen, P. J., Sato, S. & Tedder, T. F. CD19 amplifies B lymphocyte signal transduction by regulating Src-family protein tyrosine kinase activation. *J. Immunol.* **162**, 7088–7094 (1999).
26. Alfaro, A. *et al.* An alternatively spliced form of CD79b gene may account for altered B-cell receptor expression in B-chronic lymphocytic leukemia. *Blood* **93**, 2327–2335 (1999).
27. Lankester, A. C. *et al.* Antigen receptor nonresponsiveness in chronic lymphocytic leukemia B cells. *Blood* **86**, 1090–1097 (1995).
28. Gobessi, S. *et al.* ZAP-70 enhances B-cell-receptor signaling despite absent or inefficient tyrosine kinase activation in chronic lymphocytic leukemia and lymphoma B cells. *Blood* **109**, 2032–2039 (2007).
29. Wiestner, A. *et al.* ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. *Blood* **101**, 4944–4951 (2003).
30. Durig, J. *et al.* ZAP-70 expression is a prognostic factor in chronic lymphocytic leukemia. *Leukemia* **17**, 2426–2434 (2003).
31. Mockridge, C. I. *et al.* Reversible anergy of slgM-mediated signaling in the two subsets of CLL defined by VH-gene mutational status. *Blood* **109**, 4424–4431 (2007).
32. Efremov, D. G., Gobessi, S. & Longo, P. G. Signaling pathways activated by antigen-receptor engagement in chronic lymphocytic leukemia B-cells. *Autoimmun. Rev.* **7**, 102–108 (2007).
33. Allsup, D. J. *et al.* B-cell receptor translocation to lipid rafts and associated signaling differ between prognostically important subgroups of chronic lymphocytic leukemia. *Cancer Res.* **65**, 7328–7337 (2005).
34. Deaglio, S., Vaisitti, T., Aydin, S., Ferrero, E. & Malavasi, F. In-tandem insight from basic science combined with clinical research: CD38 as both marker and key component of the pathogenetic network underlying chronic lymphocytic leukemia. *Blood* **108**, 1135–1144 (2006).
35. Cockayne, D. A. *et al.* Mice deficient for the ecto-nicotinamide adenine dinucleotide glycohydrolase CD38 exhibit altered humoral immune responses. *Blood* **92**, 1324–1333 (1998).
36. Deaglio, S. *et al.* CD38/CD19: a lipid raft-dependent signaling complex in human B cells. *Blood* **109**, 5390–5398 (2007).
37. Deaglio, S. *et al.* CD38 and ZAP-70 are functionally linked and mark CLL cells with high migratory potential. *Blood* **110**, 4012–4021 (2007).
38. Muzio, M. *et al.* Constitutive activation of distinct BCR-signaling pathways in a subset of CLL patients: a molecular signature of anergy. *Blood* **112**, 188–195 (2008).
39. Veldurthy, A. *et al.* The kinase inhibitor dasatinib induces apoptosis in chronic lymphocytic leukemia cells *in vitro* with preference for a subgroup of patients with unmutated IgVH genes. *Blood* **112**, 1443–1452 (2008).
40. Garg, R. J. *et al.* Phase II study of dasatinib in patients with relapsed CLL [abstract]. *Blood* **112**, a4197 (2008).
41. Castro, J. E. *et al.* A phase II, open label study of AT-101 in combination with rituximab in patients with relapsed or refractory chronic lymphocytic leukemia. Evaluation of two dose regimens [abstract 3119]. *Blood* **110**, 917A–918A (2007).
42. Hallaert, D. Y. *et al.* c-Abl kinase inhibitors overcome CD40-mediated drug resistance in CLL [abstract 3078]. *Blood* **110**, 905A (2007).
43. Amrein, P. C. *et al.* A phase II study of dasatinib in relapsed and refractory chronic lymphocytic leukemia (CLL/SL) [abstract 3126]. *Blood* **110**, 920A (2007).
44. Hirsch, C. L., Smith-Windsor, E. L. & Bonham, K. Src family kinase members have a common response to histone deacetylase inhibitors in human colon cancer cells. *Int. J. Cancer* **118**, 547–554 (2006).
45. Michie, A. M. & Nakagawa, R. Elucidating the role of protein kinase C in chronic lymphocytic leukaemia. *Hematol. Oncol.* **24**, 134–138 (2006).
46. Alkan, S. *et al.* Survival role of protein kinase C (PKC) in chronic lymphocytic leukemia and determination of isoform expression pattern and genes altered by PKC inhibition. *Am. J. Hematol.* **79**, 97–106 (2005).
47. Abrams, S. T. *et al.* B-cell receptor signaling in chronic lymphocytic leukemia cells is regulated by overexpressed active protein kinase Cbetall. *Blood* **109**, 1193–1201 (2007).
48. Barragan, M. *et al.* Regulation of Akt/PKB by phosphatidylinositol 3-kinase-dependent and -independent pathways in B-cell chronic lymphocytic leukemia cells: role of protein kinase C $\beta$ . *J. Leukoc. Biol.* **80**, 1473–1479 (2006).
49. Ringshausen, I. *et al.* Mechanisms of apoptosis-induction by rottlerin: therapeutic implications for B-CLL. *Leukemia* **20**, 514–520 (2006).
50. Nakagawa, R., Soh, J. W. & Michie, A. M. Subversion of protein kinase C alpha signaling in hematopoietic progenitor cells results in the generation of a B-cell chronic lymphocytic leukemia-like population *in vivo*. *Cancer Res.* **66**, 527–534 (2006).
51. Wojciechowski, W., Li, H., Marshall, S., Dell'Agnola, C. & Espinoza-Delgado, I. Enhanced expression of CD20 in human tumor B cells is controlled through ERK-dependent mechanisms. *J. Immunol.* **174**, 7859–7868 (2005).
52. Jazirehi, A. R., Vega, M. I., Chatterjee, D., Goodglick, L. & Bonavida, B. Inhibition of the Raf-MEK1/2-ERK1/2 signaling pathway, Bcl-xL down-regulation, and chemosensitization of non-Hodgkin's lymphoma B cells by rituximab. *Cancer Res.* **64**, 7117–7126 (2004).
53. Marzo, I. *et al.* Farnesyltransferase inhibitor BMS-214662 induces apoptosis in B-cell chronic lymphocytic leukemia cells. *Leukemia* **18**, 1599–1604 (2004).
54. Smal, C. *et al.* Pharmacological inhibition of the MAPK/ERK pathway increases sensitivity to 2-chloro-2'-deoxyadenosine (CdA) in the B-cell leukemia cell line EHEB. *Biochem. Pharmacol.* **73**, 351–358 (2007).
55. Caligaris-Cappio, F. & Ghia, P. Novel insights in chronic lymphocytic leukemia: are we getting closer to understanding the pathogenesis of the disease? *J. Clin. Oncol.* **26**, 4497–4503 (2008).
56. Ghia, P., Granziero, L., Chilosi, M. & Caligaris-Cappio, F. Chronic B cell malignancies and bone marrow microenvironment. *Semin. Cancer Biol.* **12**, 149–155 (2002).
57. Mackus, W. J. M. *et al.* Expansion of CMV-specific CD8<sup>+</sup>CD45RA<sup>+</sup>CD27<sup>+</sup> T cells in B-cell chronic lymphocytic leukemia. *Blood* **102**, 1057–1063 (2003).
58. Pedersen, I. M. & Reed, J. C. Microenvironmental interactions and survival of CLL B-cells. *Leuk. Lymphoma* **45**, 2365–2372 (2004).
59. Burger, J. A., Burger, M. & Kippes, T. J. Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood* **94**, 3658–3667 (1999).
60. Mainou-Fowler, T., Miller, S., Proctor, S. J. & Dickinson, A. M. The levels of TNF alpha, IL4 and IL10 production by T-cells in B-cell chronic lymphocytic leukaemia (B-CLL). *Leuk. Res.* **25**, 157–163 (2001).

61. Hamblin, A. D. & Hamblin, T. J. The immunodeficiency of chronic lymphocytic leukaemia. *Br. Med. Bull.* **87**, 49–62 (2008).
62. Sinisalo, M. *et al.* Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. *Br. J. Haematol.* **114**, 107–110 (2001).
63. Serrano, D. *et al.* Clonal expansion within the CD4<sup>+</sup>CD57<sup>+</sup> and CD8<sup>+</sup>CD57<sup>+</sup> T cell subsets in chronic lymphocytic leukemia. *J. Immunol.* **158**, 1482–1489 (1997).
64. Kay, N. E., Johnson, J. D., Stanek, R. & Douglas, S. D. T-cell subpopulations in chronic lymphocytic leukemia: abnormalities in distribution and *in vitro* receptor maturation. *Blood* **54**, 540–544 (1979).
65. Totterman, T. H., Carlsson, M., Simonsson, B., Bengtsson, M. & Nilsson, K. T-cell activation and subset patterns are altered in B-CLL and correlate with the stage of the disease. *Blood* **74**, 786–792 (1989).
66. Trojan, A. *et al.* Immunoglobulin framework-derived peptides function as cytotoxic T-cell epitopes commonly expressed in B-cell malignancies. *Nat. Med.* **6**, 667–672 (2000).
67. Ranheim, E. A. & Kipps, T. J. Activated T-cells induce expression of B7/Bb1 on normal or leukemic B-cells through a Cd40-dependent signal. *J. Exp. Med.* **177**, 925–935 (1993).
68. Ramsay, A. G. *et al.* Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. *J. Clin. Invest.* **118**, 2427–2437 (2008).
69. Frolova, E. A., Scott, S. C. & Jones, R. A. Cd45Ro<sup>+</sup> T-cells immunoregulate spontaneous *in vitro* immunoglobulin production by normal and chronic lymphocytic-leukemia B-cells. *Leuk. Lymphoma* **18**, 103–111 (1995).
70. Scrivener, S., Kaminski, E. R., Demaine, A. & Prentice, A. G. Analysis of the expression of critical activation/interaction markers on peripheral blood T cells in B-cell chronic lymphocytic leukaemia: evidence of immune dysregulation. *Br. J. Haematol.* **112**, 959–964 (2001).
71. Tivol, E. A. *et al.* Loss of Ctl-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of Ctl-4. *Immunity* **3**, 541–547 (1995).
72. Luqman, M. *et al.* The antileukemia activity of a human anti-CD40 antagonist antibody, HCD122, on human chronic lymphocytic leukemia cells. *Blood* **112**, 711–720 (2008).
73. Messmer, D. & Kipps, T. J. CD154 gene therapy for human B-cell malignancies. *Ann. NY Acad. Sci.* **1062**, 51–60 (2005).
74. Biagi, E. *et al.* Molecular transfer of CD40 and OX40 ligands to leukemic human B cells induces expansion of autologous tumor-reactive cytotoxic T lymphocytes. *Blood* **105**, 2436–2442 (2005).
75. Ghia, P. *et al.* Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4<sup>+</sup>, CD40L<sup>+</sup> T cells by producing CCL22. *Eur. J. Immunol.* **32**, 1403–1413 (2002).
76. Chilosi, M. *et al.* Immunohistochemical demonstration of follicular dendritic cells in bone-marrow involvement of B-cell chronic lymphocytic-leukemia. *Cancer* **56**, 328–332 (1985).
77. Ghia, P. *et al.* The pattern of CD38 expression defines a distinct subset of chronic lymphocytic leukemia (CLL) patients at risk of disease progression. *Blood* **101**, 1262–1269 (2003).
78. Patten, P. E. M. *et al.* CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. *Blood* **111**, 5173–5181 (2008).
79. Tinhofer, I. *et al.* Expression levels of CD38 in T cells predict course of disease in male patients with B-chronic lymphocytic leukemia. *Blood* **108**, 2950–2956 (2006).
80. Deaglio, S., Aydin, S., Vaisitti, T., Bergui, L. & Malavasi, F. CD38 at the junction between prognostic marker and therapeutic target. *Trends Mol. Med.* **14**, 210–218 (2008).
81. Kretz-Rommel, A. & Bowditch, K. S. Rationale for anti-CD200 immunotherapy in B-CLL and other hematologic malignancies: new concepts in blocking immune suppression. *Expert Opin. Biol. Ther.* **8**, 5–15 (2008).
82. Lagneaux, L., Delforge, A., Bron, D., De Bruyn, C. & Stryckmans, P. Chronic lymphocytic leukemic B cells but not normal B cells are rescued from apoptosis by contact with normal bone marrow stromal cells. *Blood* **91**, 2387–2396 (1998).
83. Ogata, T., Yamakawa, M., Imai, Y. & Takahashi, T. Follicular dendritic cells adhere to fibronectin and laminin fibers via their respective receptors. *Blood* **88**, 2995–3003 (1996).
84. Ansel, K. M. *et al.* A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* **406**, 309–314 (2000).
85. Trentin, L. *et al.* Homeostatic chemokines drive migration of malignant B cells in patients with non-Hodgkin lymphomas. *Blood* **104**, 502–508 (2004).
86. Burger, M. *et al.* Small peptide inhibitors of the CXCR4 chemokine receptor (CD184) antagonize the activation, migration, and antiapoptotic responses of CXCL12 in chronic lymphocytic leukemia B cells. *Blood* **106**, 1824–1830 (2005).
87. Tiili, K. J. *et al.* CLL, but not normal, B cells are dependent on autocrine VEGF and alpha(4) beta(1) integrin for chemokine-induced motility on and through endothelium. *Blood* **105**, 4813–4819 (2005).
88. Alfonso-Perez, M. *et al.* Anti-CCR7 monoclonal antibodies as a novel tool for the treatment of chronic lymphocyte leukemia. *J. Leukoc. Biol.* **79**, 1157–1165 (2006).
89. List, A. F. Lenalidomide—the phoenix rises. *N. Engl. J. Med.* **357**, 2183–2186 (2007).
90. Richardson, P. Management of the relapsed/refractory myeloma patient: Strategies incorporating lenalidomide. *Semin. Hematol.* **42** (4 Suppl. 4), S9–S15 (2005).
91. Mitsiades, C. S., Hayden, P. J., Anderson, K. C. & Richardson, P. G. From the bench to the bedside: emerging new treatments in multiple myeloma. *Best Pract. Res. Clin. Haematol.* **20**, 797–816 (2007).
92. Chanan-Khan, A. *et al.* Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. *J. Clin. Oncol.* **24**, 5343–5349 (2006).
93. Ferrajoli, A. *et al.* Lenalidomide is active in patients with relapsed/refractory chronic lymphocytic leukemia (CLL) carrying unfavorable chromosomal abnormalities [abstract]. *Blood* **110**, 754A (2007).
94. Andritsos, L. A. *et al.* Higher doses of lenalidomide are associated with unacceptable toxicity including life-threatening tumor flare in patients with chronic lymphocytic leukemia. *J. Clin. Oncol.* **26**, 2519–2525 (2008).
95. Carlo-Stella, C. *et al.* Targeting TRAIL agonistic receptors for cancer therapy. *Clin. Cancer Res.* **13**, 2313–2317 (2007).
96. Adams, J. M. & Cory, S. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. *Curr. Opin. Immunol.* **19**, 488–496 (2007).
97. Tinhofer, I. *et al.* Inversion of CD4<sup>+</sup>/CD8<sup>+</sup> ratio in B chronic lymphocytic leukemia correlates with differential sensitivity of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes to the killing efficacy of Fas (APO-1/CD95) ligand(+) tumor cells. *Blood* **92**, 271B (1998).
98. Proto-Siqueira, R. *et al.* SAGE analysis demonstrates increased expression of TOSO contributing to Fas-mediated resistance in CLL. *Blood* **112**, 394–397 (2008).
99. Chu, P. *et al.* Latent sensitivity to Fas-mediated apoptosis after CD40 ligation may explain activity of CD154 gene therapy in chronic lymphocytic leukemia. *Proc. Natl Acad. Sci. USA* **99**, 3854–3859 (2002).
100. Anether, G., Tinhofer, I., Senfter, M. & Greil, R. Tetrocarcin-A-induced ER stress mediates apoptosis in B-CLL cells via a Bcl-2-independent pathway. *Blood* **101**, 4561–4568 (2003).
101. Kater, A. P. *et al.* Inhibitors of XIAP sensitize CD40-activated chronic lymphocytic leukemia cells to CD95-mediated apoptosis. *Blood* **106**, 1742–1748 (2005).
102. Dicker, F., Kater, A. P., Fukuda, T. & Kipps, T. J. Fas-ligand (CD178) and TRAIL synergistically induce apoptosis of CD40-activated chronic lymphocytic leukemia B cells. *Blood* **105**, 3193–3198 (2005).
103. Inoue, S. *et al.* Histone deacetylase inhibitors potentiate TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in lymphoid malignancies. *Cell Death Differ.* **11** (Suppl. 2), S193–S206 (2004).
104. MacFarlane, M. *et al.* Chronic lymphocytic leukemic cells exhibit apoptotic signaling via TRAIL-R1. *Cell Death Differ.* **12**, 773–782 (2005).
105. Hallaert, D. Y. *et al.* c-Abl kinase inhibitors overcome CD40-mediated drug resistance in CLL: implications for therapeutic targeting of chemoresistant niches. *Blood* **112**, 5141–5149 (2008).
106. Smit, L. A. *et al.* Differential Noxa/Mcl-1 balance in peripheral versus lymph node chronic lymphocytic leukemia cells correlates with survival capacity. *Blood* **109**, 1660–1668 (2007).
107. Willimott, S., Baou, M., Nares, K. & Wagner, S. D. CD154 induces a switch in pro-survival Bcl-2 family members in chronic lymphocytic leukaemia. *Br. J. Haematol.* **138**, 721–732 (2007).
108. Mackus, W. J. M. *et al.* Chronic lymphocytic leukemia cells display p53-dependent drug-induced Puma upregulation. *Leukemia* **19**, 427–434 (2005).
109. Stankovic, T. *et al.* Microarray analysis reveals that TP53-and ATM-mutant B-CLLs share a defect in activating proapoptotic responses after DNA damage but are distinguished by major differences in activating prosurvival responses. *Blood* **103**, 291–300 (2004).
110. Morales, A. A. *et al.* Expression and transcriptional regulation of functionally distinct Bmf isoforms in B-chronic lymphocytic leukemia cells. *Leukemia* **18**, 41–47 (2004).
111. Datta, S. R. *et al.* Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* **91**, 231–241 (1997).

112. Qi, X. J., Wildey, G. M. & Howe, P. H. Evidence that Ser(87) of Bim(EL) is phosphorylated by Akt and regulates Bim(EL) apoptotic function. *J. Biol. Chem.* **281**, 813–823 (2006).
113. Pekarsky, Y. *et al.* Tcl1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. *Cancer Res.* **66**, 11590–11593 (2006).
114. Calin, G. A. *et al.* MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc. Natl Acad. Sci. USA* **105**, 5166–5171 (2008).
115. Cimmino, A. *et al.* miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl Acad. Sci. USA* **102**, 13944–13949 (2005).
116. Gowda, A. *et al.* IL-21 mediates apoptosis through up-regulation of the BH3 family member BIM and enhances both direct and antibody-dependent cellular cytotoxicity in primary chronic lymphocytic leukemia cells *in vitro*. *Blood* **111**, 4723–4730 (2008).
117. Villunger, A. *et al.* p53- and drug-induced apoptotic responses mediated by BH3-only proteins Puma and Noxa. *Science* **302**, 1036–1038 (2003).
118. Zenz, T. *et al.* Monoallelic TP53 inactivation is associated with poor prognosis in chronic lymphocytic leukemia: results from a detailed genetic characterization with long-term follow-up. *Blood* **112**, 3322–3329 (2008).
119. Austen, B. *et al.* Mutation status of the residual ATM allele is an important determinant of the cellular response to chemotherapy and survival in patients with chronic lymphocytic leukemia containing an 11q deletion. *J. Clin. Oncol.* **25**, 5448–5457 (2007).
120. Gryshchenko, I. *et al.* MDM2 SNP309 is associated with poor outcome in B-cell chronic lymphocytic leukemia. *J. Clin. Oncol.* **26**, 2252–2257 (2008).
121. Yin, X. M. *et al.* Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* **400**, 886–891 (1999).
122. Dicker, F. *et al.* CD154 induces p73 to overcome the resistance to apoptosis of chronic lymphocytic leukemia cells lacking functional p53. *Blood* **108**, 3450–3457 (2006).
123. O'Brien, S. *et al.* Randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen sodium (Bcl-2 antisense) in patients with relapsed or refractory chronic lymphocytic leukemia. *J. Clin. Oncol.* **25**, 1114–1120 (2007).
124. O'Brien, S. M. *et al.* Phase I to II multicenter study of oblimersen sodium, a Bcl-2 antisense oligonucleotide, in patients with advanced chronic lymphocytic leukemia. *J. Clin. Oncol.* **23**, 7697–7702 (2005).
125. Labi, V., Grespi, F., Baumgartner, F. & Villunger, A. Targeting the Bcl-2-regulated apoptosis pathway by BH3 mimetics: a breakthrough in anticancer therapy? *Cell Death Differ.* **15**, 977–987 (2008).
126. Oltschendorf, T. *et al.* An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**, 677–681 (2005).
127. Trudel, S. *et al.* Preclinical studies of the pan-Bcl inhibitor obatoclax (GX015–070) in multiple myeloma. *Blood* **109**, 5430–5438 (2007).
128. Nguyen, M. *et al.* Small molecule obatoclax (GX15–070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. *Proc. Natl Acad. Sci. USA* **104**, 19512–19517 (2007).
129. O'Brien, S. M. *et al.* Phase I study of obatoclax mesylate (GX15–070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. *Blood* **113**, 299–305 (2008).
130. Wilson, W. H. *et al.* A phase 1 study evaluating the safety, pharmacokinetics, and efficacy of ABT-263 in subjects with refractory or relapsed lymphoid malignancies [abstract]. *J. Clin. Oncol.* **26**, a8511 (2008).
131. Balakrishnan, K., Burger, J. A., Wierda, W. G. & Gandhi, V. AT-101 induces apoptosis in CLL B-cells and overcomes stromal cell-mediated Mcl-1 induction and drug resistance. *Blood* **113**, 149–153 (2008).
132. Aloyz, R. *et al.* Imatinib sensitizes CLL lymphocytes to chlorambucil. *Leukemia* **18**, 409–414 (2004).
133. Mansour, A., Chang, V. T., Srinivas, S., Harrison, J. & Raveche, E. Correlation of ZAP-70 expression in B cell leukemias to the *ex vivo* response to a combination of fludarabine/genistein. *Cancer Immunol. Immunother.* **56**, 501–514 (2007).
134. Ganeshaguru, K. *et al.* Actions of the selective protein kinase C inhibitor PKC412 on B-chronic lymphocytic leukemia cells *in vitro*. *Haematologica* **87**, 167–176 (2002).
135. Varterasian, M. L. *et al.* Phase II trial of bryostatin 1 in patients with relapsed low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Clin. Cancer Res.* **6**, 825–828 (2000).
136. Roberts, J. D. *et al.* Phase I study of bryostatin 1 and fludarabine in patients with chronic lymphocytic leukemia and indolent (non-Hodgkin's) lymphoma. *Clin. Cancer Res.* **12**, 5809–5816 (2006).
137. Galli, U. *et al.* Synthesis and biological evaluation of isosteric analogues of FK866, an inhibitor of NAD salvage. *ChemMedChem.* **3**, 771–779 (2008).
138. Rhodes, N. *et al.* Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity. *Cancer Res.* **68**, 2366–2374 (2008).
139. Zeng, Z. *et al.* Inhibition of CXCR4 with the novel RCP168 peptide overcomes stroma-mediated chemoresistance in chronic and acute leukemias. *Mol. Cancer Ther.* **5**, 3113–3121 (2006).
140. Cornall, R. J. *et al.* Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity* **8**, 497–508 (1998).
141. Xu, Y., Beavitt, S. J., Harder, K. W., Hibbs, M. L. & Tarlinton, D. M. The activation and subsequent regulatory roles of Lyn and CD19 after B cell receptor ligation are independent. *J. Immunol.* **169**, 6910–6918 (2002).

#### Acknowledgments

We thank Petra Desch for skillful support with graphical presentation. The work of the authors is supported by FWF grants L488-B13, P19481-B12, and SFB 021-P11 and grants of the Province of Salzburg.